Electronic Supplementary Material (ESI) for Biomaterials Science.

Supplementary Data

IL-10-secreting human MSCs generated by TALEN gene editing ameliorate liver fibrosis through enhanced anti-fibrotic activity

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Supplemental material and method

Fluorescence-activated cell sorting (FACS) analysis

FACS analysis was performed as previously reported [1]. Cells were resuspended in Dulbecco's PBS (Cellgro, Manassas, MD) and incubated for 20 min at 4°C either directly with FITC-conjugated Abs. The antibodies used were anti-TNF-α (from R&D system). The corresponding isotype-identical IgGs served as negative controls. After cell staining, quantitative FACS was performed on a FACStar flow cytometer (BD).

Conditioned Media (CM) Preparation

AMM and AMM/I ($5x10^6$ cells each) were seeded into T-175 cell culture flasks and grown in normal medium or low-glucose DMEM (Gibco) containing 10% FBS, 100 U/mL penicillin and 100 mg/ mL streptomycin (Gibco) for 48 h until the cells reached approximately 80% confluence. Culture media from each sample was then centrifuged at 1,0006g for 10 min and the supernatants were collected and used as CM for the study.

CM injection into liver

CM injection was performed as previously reported [2] .CM was concentrated 25-fold using ultrafiltration units (Millipore, Bedford, MA) and 0.5 ml was injected into portal vein.

Isolation of $CD4^+$ and $F4/80^+$ cells

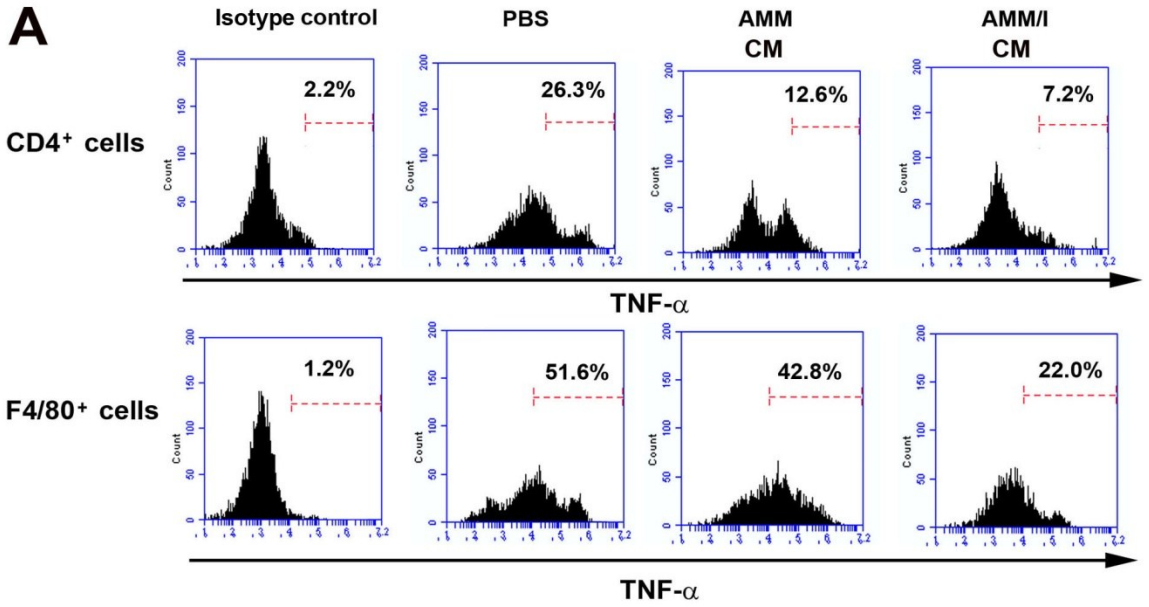
The liver tissues were harvested from TAA treated mice one week after CM injection. And then liver tissues were minced and digested at 37°C for 45 - 60 min with an enzyme cocktail (Collagenase A, Elastase, and DNase I, Roche Applied Science). Single cell suspensions were prepared by filtering through a 30-μm strainer and samples were resuspended in MACS buffer blocking reagent. Mouse CD4 and F4/80 microbeads (Miltenyi Biotec) were sequentially added and incubated at 4°C for 15 minutes. The cells were washed and resuspended in MACS buffer. $CD4^+$ and $F4/80^+$ cells were isolated using magnetic LS columns (Miltenyi Biotec) according to the manufacturer's instructions.

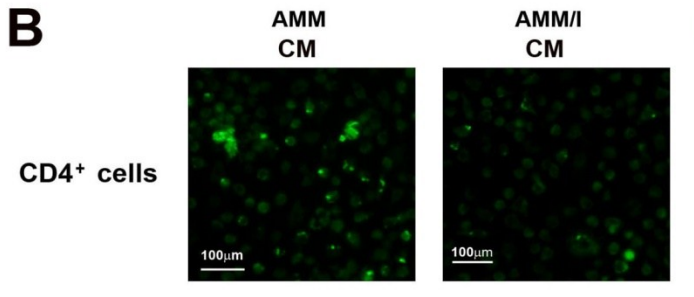
Quantification of cell engraftment in liver tissue

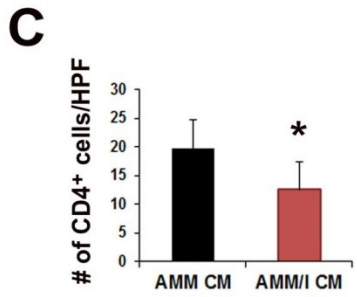
Cell engraftment in the fibrotic liver was quantified by histological analyses. Briefly, five fields from 10 tissue sections were randomly selected, and the number of cells was counted in each field.

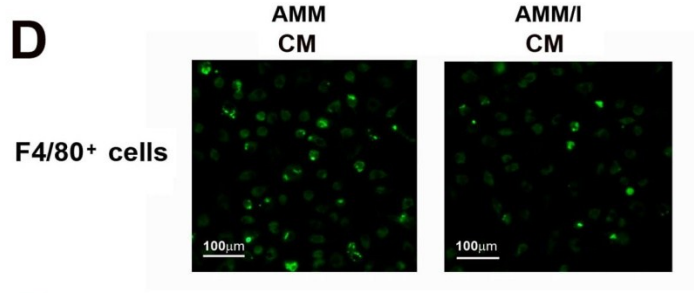
Supplementary Figure Legends

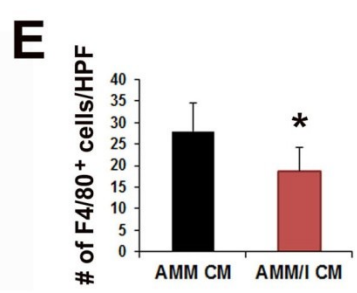
Supplementary Figure 1. *In vivo* anti-inflammatory potential of AMM/I CM. (A) FACS analysis showed that treatment of AMM/I CM reduced the expression of TNF- a in $CD4^+$ T cells or $F4/80^+$ macrophage. (B, D) Immunocytochemistry analysis also showed that treatment of AMM/I CM reduced the expression of TNF-a in $CD4^+$ T cells or $F4/80^+$ macrophage. Cells were stained with TNF-a (Green florescent) for 1 hr. (E, F) Quantification of TNF-a positive cells. HPF=high power field. $^{**}p < 0.01$, n = 5 per group. (F) Representative immunohistochemistry images of infiltrated macrophage in fibrotic liver. **Treatment of AMM/I CM suppressed infiltrating macrophage. F4/80(red) and** nuclei (blue) were stained with DAPI. (G) Quantification of F4/80 positive cells. HPF=high power field. $^{**}p < 0.01$, $^{*}p < 0.05$, n = 5 per group. (H) Levels of mRNA expression were quantified by RT-PCR. $^{**}p < 0.01$, $^{*}p < 0.05$, n = 5 per group.

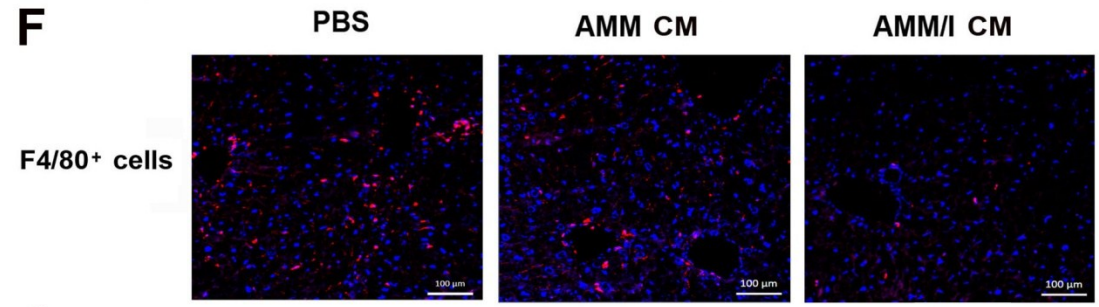

A
Isotype control
PBS
AMM CM
AMM/I CM
CD4+ cells
2.2%
26.3%
12.6%
7.2%
Count
TNF-α
F4/80+ cells
1.2%
51.6%
42.8%
22.0%
Count
TNF-α

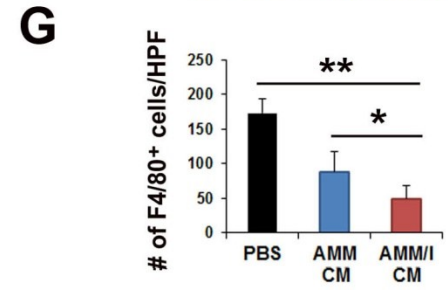

B
AMM CM
AMM/I CM
CD4+ cells
100μm
100μm

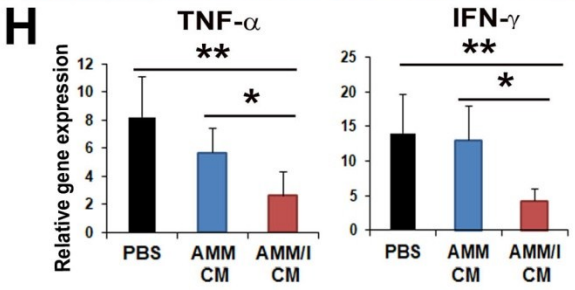

C
# of CD4+ cells/HPF
30
25
20
15
10
5
0
*
AMM CM
AMM/I CM


D
AMM CM
AMM/I CM
F4/80+ cells
100μm
100μm


E
# of F4/80+ cells/HPF
40
35
30
25
20
15
10
5
0
*
AMM CM
AMM/I CM


F
PBS
AMM CM
AMM/I CM
F4/80+ cells
100 μm
100 μm
100 μm


G
# of F4/80+ cells/HPF
250
200
150
100
50
0
**
*
PBS
AMM CM
AMM/I CM


H
TNF-α
IFN-γ
Relative gene expression
12
10
8
6
4
2
0
**
*
25
20
15
10
5
0
**
*
PBS
AMM CM
AMM/I CM
PBS
AMM CM
AMM/I CM

References

[1] Kim MH, Zhang HZ, Kim SW. Combined growth factors enhanced angiogenic potential of cord blood-derived mononuclear cells transplanted to ischemic limbs. Journal of molecular and cellular cardiology. 2011;51:702-12.

[2] van Poll D, Parekkadan B, Cho CH, Berthiaume F, Nahmias Y, Tilles AW, et al. Mesenchymal stem cell-derived molecules directly modulate hepatocellular death and regeneration in vitro and in vivo. Hepatology. 2008;47:1634-43.